



Development of phytoplankton communities and common off-flavors in a biofloc technology system used for the culture of channel catfish (*Ictalurus punctatus*)

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ABSTRACT

The use of biofloc technology production systems continues to increase in the aquaculture industry worldwide. Recent research demonstrated that outdoor biofloc systems can be used to produce high yields of channel catfish (*Ictalurus punctatus*). However, studies have not yet been performed to determine the development and composition of phytoplankton communities and related off-flavor problems in these biofloc production systems. In this study, water samples were collected biweekly from May to November and channel catfish samples were collected during harvest in November from nine 18.6 m² biofloc culture tanks. Water and fillet samples were analyzed for levels of the common off-flavor compounds geosmin and 2-methylisoborneol (MIB). The development and composition of phytoplankton communities in each culture tank was also monitored. In addition, water and biofloc samples were evaluated to assess the microbial sources of geosmin and MIB within the culture tanks. Phytoplankton (including algae and cyanobacteria attached to bioflocs) biomass, as determined by concentrations of chlorophyll *a* in the water, gradually increased in all tanks over time. Phytoplankton communities that developed in the culture tanks were dominated by fast-growing, unicellular and small colonial types of green algae (chlorophytes) and diatoms (bacillariophytes) and slower growing, small colonial types of cyanobacteria (cyanophytes). A positive correlation ($p < 0.05$) between cumulative feed addition and chlorophyll *a* concentration was found. Although geosmin and MIB were present in the culture water of each tank during most of the study, levels were typically low and only one tank yielded catfish with geosmin and MIB in their flesh at levels high enough to be designated as off-flavor. A positive correlation ($p < 0.05$) between cumulative feed addition and MIB concentrations in the water of culture tanks indicates a greater potential for MIB-related off-flavor problems at high feed application rates. The microbial sources responsible for production of geosmin and MIB in the culture tanks remain unknown.

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1. Introduction

Aquaculturists continue to increase their interest in and use of mixed suspended-growth production systems, also referred to as biofloc technology (BFT) systems, for culturing various aquatic animals. These BFT systems rely on the living microorganisms in the biofloc (composed of microbial biomass and particulate organic matter) maintained in the water column to assist in ammonia removal via phytoplankton and bacterial uptake and bacterial oxidation of ammonia-N (NH₃-N) to nitrite-N (NO₂-N) and then subsequent oxidation of NO₂-N to nitrate-N (NO₃-N) during nitrification (Brune et al., 2003; Ebeling et al., 2006; Hargreaves, 2006).

Therefore, these biological processes play a critical role in reducing ammonia and nitrite to levels below those that can be toxic or growth-limiting for cultured finfish.

Currently, most channel catfish (*Ictalurus punctatus*) production in the southeastern United States of America is conducted in earthen, embankment-type ponds. In these earthen ponds, phytoplankton will assimilate and reduce NH₃-N concentrations in the pond waters (Hargreaves, 2006). Cyanobacteria (blue-green algae) usually dominate the phytoplankton communities in catfish ponds because of their ability to regulate cell buoyancy by collapse and reformation of intracellular gas vesicles in the poorly mixed and often stratified water column; this physiological mechanism allows cyanobacteria to outcompete other types of phytoplankton for sunlight (Paerl and Tucker, 1995). Certain species of cyanobacteria are undesirable due to their production of odorous compounds that can accumulate in the flesh of fish

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and subsequently result in an “off-flavor” and unmarketable product. The most common microbial-produced off-flavor metabolites in catfish aquaculture ponds are geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (MIB) [(1-*R*-*exo*)-1,2,7,7-tetramethylbicyclo[2.2.1]heptan-2-ol], which can accumulate in the flesh of the catfish and cause “earthy” and “musty” off-flavors, respectively (Tucker, 2000).

Geosmin and MIB production in catfish aquaculture ponds are commonly associated with certain planktonic species of cyanobacteria. In catfish ponds located in Mississippi and Alabama, USA, *Planktothrix perornata* f. *attenuata* [Skuja] (Anagnostidis and Komárek, 1988) [formerly designated as *Oscillatoria perornata* f. *attenuata* (Skuja, 1949)] has been attributed as the main cyanobacterial source for MIB-related off-flavor in channel catfish (van der Ploeg et al., 1995; Schrader and Dennis, 2005). Geosmin production in catfish ponds is commonly attributed to certain cyanobacterial species of *Anabaena* (Tucker, 2000). Although actinomycetes (filamentous bacteria) isolated from aquaculture pond sediments have also been identified as producers of geosmin and MIB (Schrader and Blevins, 1993), they are now considered to be minor contributors to these common off-flavor problems in pond-cultured catfish (Tucker, 2000).

Recent research evaluated and demonstrated the effectiveness of using an outdoor BFT production system to produce high yields of channel catfish when stocking up to 0.14 kg/m³ (Green, 2010). However, previous research has not evaluated the types and occurrences of environmentally derived off-flavors in catfish raised in outdoor BFT culture units. Because the tanks used in biofloc systems are continuously well-mixed and do not contain an earthen sediment bottom, conditions may be less favorable for the growth of cyanobacterial species commonly associated with geosmin and MIB production in catfish aquaculture ponds. However, detailed studies of phytoplankton communities and their relationship to potential off-flavor problems in freshwater BFT systems have not been performed. Off-flavor problems in pond-based systems for the culture of channel catfish have been estimated to cost producers as much as US \$60 million annually (Tucker, 2000). Therefore, if the incidences of common off-flavor episodes are significantly reduced in BFT systems, they may prove to be economical for large-scale commercial production.

In this study, water and catfish fillet samples were analyzed to determine the presence and levels of geosmin and MIB in relation to their potential contribution to preharvest off-flavors. Phytoplankton community structure and biofloc samples were evaluated to assist in the determination of potential microbial sources of geosmin and MIB within the BFT culture units. In addition, potential correlations between geosmin and MIB concentrations in the water of BFT tanks and other measured variables (e.g., cumulative feed addition and chlorophyll *a* concentration) were determined.

2. Materials and methods

2.1. Biofloc technology production system

This current study on off-flavor and phytoplankton community development in the BFT tanks was supplementary to another study. In that study, five levels of initial channel catfish biomass were selected to examine the relationship between initial biomass and net yield in the BFT systems.

Nine wood-framed tanks (18.6 m², mean 15.6 m³ of water, mean depth of 0.81 m) lined with high density polyethylene (HDPE) and located at the USDA Agricultural Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center (SNARC), Stuttgart, Arkansas, were used for this study. One 1.865-kW blower/3 tanks provided air continuously through a diffuser grid on the bottom

of each tank. Tanks were filled with well water (total alkalinity = 228.4 mg/L as CaCO₃) on 4–30–10. During the first two weeks of May, each tank was seeded with 2.5 m³ of water from a SNARC pond containing an algal bloom, fertilized with 0.28 kg 11–37–0 (N–P–K) and 1.8 kg dried molasses (Sweet45; Westway Feed Products, New Orleans, LA), and treated with 3.4 kg stock salt to ensure that chloride concentration exceeded 100 mg/L. Pond water was added to the tanks in an attempt to establish the growth of phytoplankton to aid in the removal of ammonia-N from the culture water.

2.2. Catfish stocking and feeding rates

Fingerling channel catfish (mean weight = 78.2 g) were stocked into tanks on 5–13–10. Initial fish biomasses were 0.4 kg/m³ (*N* = 3), 0.5 kg/m³ (*N* = 1), 0.9 kg/m³ (*N* = 3), 1.4 kg/m³ (*N* = 1), and 2.5 kg/m³ (*N* = 1). Each stocking level was assigned randomly to one tank, except for two stocking levels (0.4 and 0.9 kg/m³; selected based upon previously unpublished research by B.W. Green) that were assigned randomly to three tanks.

Fish in each tank were fed daily as much 32% protein floating extruded feed (ARKAT Nutrition, Dumas, AR) as they could consume in 10 min. Daily feed rates during this study were high and within the reported range of feed rates for biofloc systems. Daily feed rate during the peak growing season (late June through early October) averaged 60.9, 43.1, 69.5, 79.1, and 100.7 g/m³ per week in tanks stocked with an initial fish biomass of 0.4, 0.5, 0.9, 1.4, and 2.5 kg/m³, respectively. Total feed fed over the entire study period was 8.25, 5.76, 9.42, 10.39, and 13.49 kg/m³, respectively. Catfish were fed up to one day prior to harvest. The range of mean daily feed rates that we report for the peak feed consumption period (27 June–9 October) are equivalent to 323 lb/acre-day (43.1 g feed/m³-day × 15.6 m³/tank × tank/18.6 m² × 4049 m²/acre × kg/1000 g × 2.2046 lb/kg) for the 0.5 kg/m³ stocking rate and up to 754 lb/acre-day for the 2.5 kg/m³ stocking rate. The mean daily feed application on a volumetric basis relative to a commercial catfish pond can also be calculated. The typical commercial catfish pond averages 1.37 m deep (USDA, 2003); thus, a 1-acre pond would contain 5547 m³ (4049 m²/acre × 1.37 m). The range of mean daily feeding rates that we report for the peak feed consumption period (27 June–9 October) are equivalent to 527 lb/acre-day (60.9 g feed/m³-day × 5547 m³/acre pond × kg/1000 g × 2.2046 lb/kg) for the 0.5 kg/m³ stocking rate to 1231 lb/acre-day for the 2.5 kg/m³ stocking rate. Given the nature of this production system, we feel that reporting feed input and yield on a “per cubic meter” basis is more appropriate than reporting on a “per acre” basis.

Dried molasses, as a carbon source for microorganisms, was added to individual tanks through the end of June according to Avnimelech (1999) in order to counter high total ammonia-nitrogen concentrations. The tanks were operated with zero water exchange; however, water was added as needed to replace evaporative losses. Tank water level was maintained 5–10 cm below the top of the drain pipe in order to capture rainwater. Fish were harvested by draining tanks on 11–10–10.

2.3. General water quality

Water samples were collected weekly from each tank. Chlorophyll *a* was extracted in 2:1 chloroform:methanol from phytoplankton (for this study, “phytoplankton” includes planktonic algae and cyanobacteria as well as those attached to bioflocs) previously filtered from water samples by using a 0.45-μm pore size glass fiber filter, and the chlorophyll *a* concentration in the extract was determined by spectroscopy (Lloyd and Tucker, 1988). Nitrite-nitrogen (NO₂-N, diazotization), nitrate-nitrogen (NO₃-N, cadmium reduction), and soluble reactive phosphorus (ascorbic acid method) were analyzed using flow injection analysis accord-

ing to manufacturer instructions (FIALab 2500; FIALab Instruments, Bellevue, WA). Total ammonia-nitrogen (TAN) was analyzed fluorometrically using the *o*-phthaldialdehyde method in a flow injection system (Genfa and Dasgupta, 1989). Beginning on 5-24-10, settleable solids, total suspended solids, and total volatile solids were measured using the methods of Eaton et al. (2005). On five occasions throughout the experiment, total alkalinity was measured by titration (Eaton et al., 2005). Sample pH was measured electrometrically. Sodium bicarbonate was added to tanks as needed to maintain pH values between 7.0 and 7.8. The dissolved oxygen (DO) concentration and water temperature in each tank were monitored continuously by a galvanic oxygen sensor (Type III, Oxyguard, Birkerød, Denmark) and a thermister (Model 109, Campbell Scientific, Logan, UT) connected to a datalogger (Model CR206 or CR10X, Campbell Scientific, Logan, UT).

2.4. Sample collection and processing

Composite water samples (1 L per tank) were collected biweekly beginning on 5-20-10 and through 11-9-10 by combining four 250-mL samples obtained approximately 6 cm below the water surface and from the middle of each side of the tank. A portion of each composite water sample was used to completely fill separate 20-mL glass scintillation vials which were stored at 4 °C until overnight shipment to the USDA-ARS-NPURL laboratory for analysis of geosmin and MIB.

At the end of the study (11-10-10), five catfish were selected at random from each tank, euthanized immediately by cranial percussion, and filleted. Catfish fillets (one fillet/fish) were placed in individual plastic bags, vacuum sealed, and immediately frozen until overnight shipment to the USDA-ARS-NPURL laboratory for analysis of geosmin and MIB levels. Fish fillets were maintained frozen until further processing to obtain microwave distillates. For analysis of each fillet, a single 20-g sample was obtained from the anterior end of the fillet by cutting 1-cm wide portions (2–3 portions per fillet) vertically from the dorsal to the ventral side of the fillet, and then each 1-cm wide sample was cut into approximately 1-cm cube-like pieces to undergo microwave distillation using the procedures of Lloyd and Grimm (1999).

2.5. Determination of geosmin and MIB levels

Prior to analysis, water samples and microwave distillates of catfish fillet samples were processed by placing 0.6-mL aliquots into separate 2-mL glass crimp-top vials and adding 0.3 g sodium chloride to each vial. The procedures of Lloyd et al. (1998) and as modified by Schrader et al. (2003) were used to quantify geosmin and MIB using solid phase microextraction and gas chromatography–mass spectrometry (SPME–GC–MS). Samples were analyzed using an Agilent 6890 gas chromatograph (Agilent, Palo, Alto, CA) and Agilent 5973 mass selective detector with attached CombiPal autosampler and solid phase microextraction assembly (LEAP Technologies, Inc., Carrboro, NC). The GC–MS conditions were the same as those outlined by Schrader et al. (2010), and each sample was run in triplicate.

2.6. Algal and cyanobacterial identification and enumeration

A second sub-sample (50 mL) was removed from the original composite water sample that was collected from each tank for geosmin and MIB analysis. Formalin (2 mL) was added to each 50-mL sample to preserve algae and cyanobacteria until microscopic examination could be performed. For each 50-mL sub-sample, identification and enumeration was performed by transferring a 1-mL sample into a Sedgewick–Rafter counting chamber and using a phase contrast microscope (150× magnification) with a Whip-

ple grid in the ocular lens. Natural units (single cells, colonies, and trichomes) were counted in five grid quadrants (fields) that were chosen at random across the counting chamber. Identification of algae and cyanobacteria was made to the genus level, with two exceptions as follows: (1) cyanobacteria commonly responsible for noxious algal blooms (Paerl and Tucker, 1995) were identified to the species level based upon the descriptions provided by Cocke (1967) and with additional reference to Desikachary (1959); and (2) members of Bacillariophyta were designated to either one of two orders [Centrales (centric) or Pennales (pennate) diatoms] or to the genus level of *Melosira* as appropriate. Reference was also made to Prescott (1962). Current taxonomic names [<http://www.algaebase.org/>] (accessed March, 2011) of phytoplankton were used. Phytoplankton abundance was reported as natural units per milliliter.

2.7. Isolation of actinomycetes

Biofloc samples (10–20 g filtrate per tank) were collected, placed in separate 20-mL glass scintillation vials, and held at 4 °C until shipment to the USDA-ARS-NPURL laboratory. Biofloc samples were serially diluted (1:10) in 0.85% saline blanks, and diluted biofloc samples (10^4 , 10^5 , and 10^6 dilutions) were used to inoculate 1% yeast extract–1% dextrose (YD) agar (pH 7.5) plates and actinomycete isolation agar (AI) (Bacto; Becton, Dickinson and Co., Franklin Lakes, NJ) plates by the spread-plate technique. Inoculated plates were incubated for 7–10 days at 29 °C.

Colonies bearing resemblance to actinomycete colony morphology (e.g., chalky appearance, “biting” into the agar surface) were streaked for isolation onto the same type of agar on which they originally grew. Isolated actinomycete colonies were aseptically removed from the agar surface and transferred to a 2-mL glass crimp-top vial containing 0.6 mL of ultrapure water and 0.3 g of sodium chloride. These vials were then analyzed by SPME–GC–MS to detect geosmin and/or MIB production by the actinomycete isolate.

2.8. Data analysis

Data of mean geosmin and MIB concentrations, chlorophyll *a* concentrations, and cumulative feed additions (g/m^3) were analyzed by correlation analysis in order to determine significant relationships ($p < 0.05$) between the measured variables. Pearson correlation analysis was performed on the data using SigmaPlot for Windows version 11.0 (Systat Software, Inc., Chicago, IL).

3. Results and discussion

Prior to the addition of the pond water to the biofloc tanks, microscopic evaluation of the pond water was performed and revealed the presence of several species of diatoms (division Bacillariophyta) and green algae (division Chlorophyta) including chlorophytes in the following genera: *Ankistrodesmus*, *Chlamydomonas*, *Closterium*, *Quadrigula*, and *Selenastrum*. No undesirable species of cyanobacteria (e.g., colonial such as potential toxin-producing *Microcystis* spp. or filamentous types such as *P. perornata*) were observed to be present in the pond water. Therefore, the pond water was selected for addition to the biofloc tanks in an attempt to provide “inocula” to establish the composition of preferred types (e.g., green algae) of phytoplankton to subsequently play a significant role in the uptake (removal) of ammonia-N, especially as feeding rates increase during grow-out of catfish in the biofloc tanks.

Overall, the dominance among the different phytoplankton groups (cyanobacteria, green algae, diatoms, and euglenoids) was variable among the biofloc tanks (Fig. 1). During the early portion

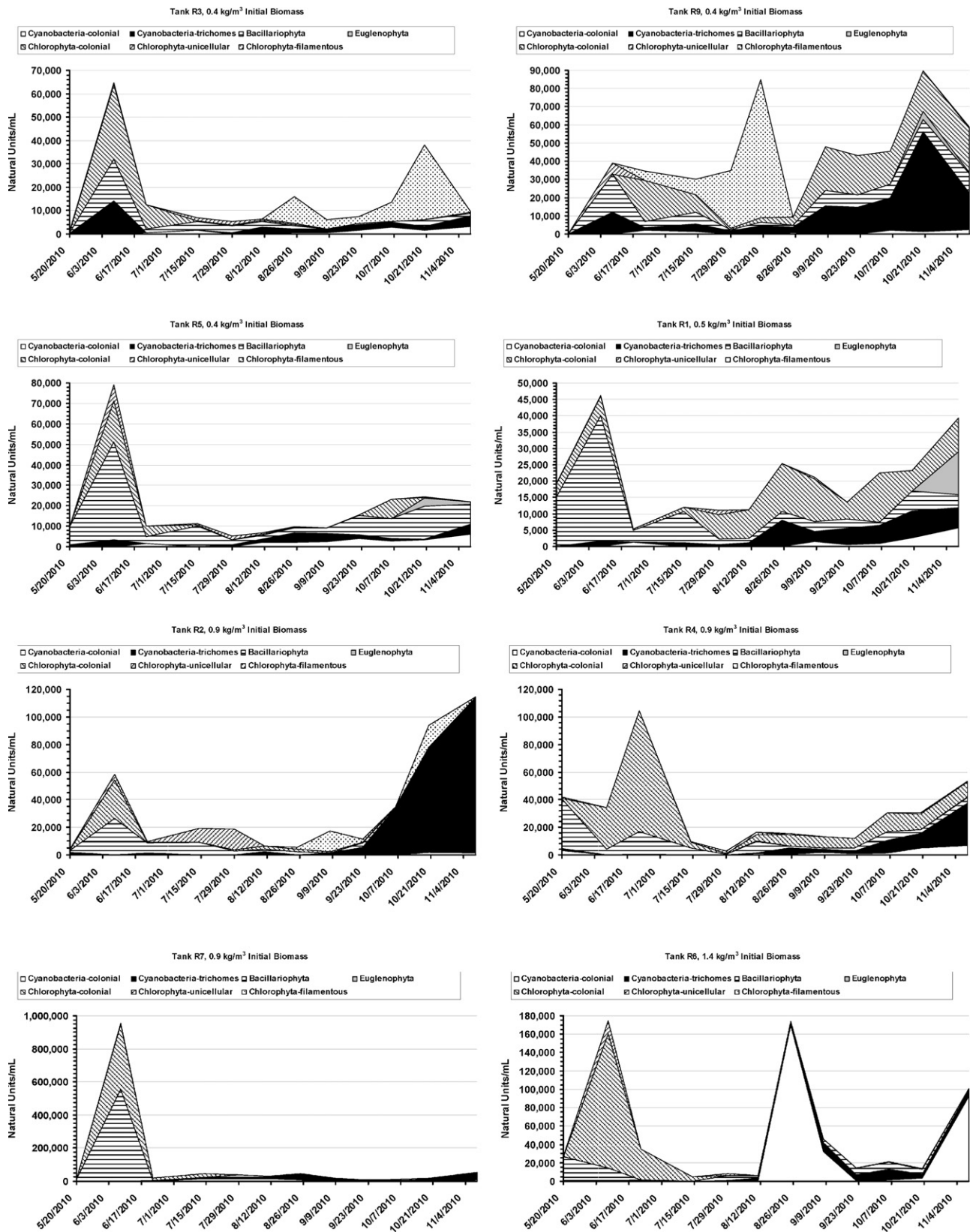


Fig. 1. Phytoplankton community composition by functional group in biofloc technology (BFT) production system culture units (15.6 m³ HDPE-lined tanks) stocked with channel catfish at initial biomasses ranging from 0.4 to 2.5 kg/m³. Note that for clarity different scales were used for the y-axis in each figure.

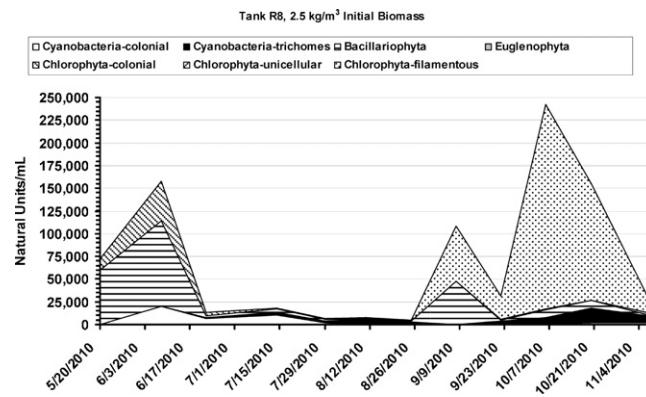


Fig. 1. (continued)

of the sampling period (5-20-10 to 7-2-10), the numbers of green algae and diatoms were highest in most tanks while the patterns of phytoplankton dominance differed among the tanks throughout the remainder of the study. Five genera of chlorophytes, two genera of cyanobacteria, and diatoms were the most common throughout the study (Table 1). Often, only one to two algal or cyanobacterial genera dominated a tank population on any given sample date. Many of the genera of green algae are unicellular, small colonial types, and these are typically fast-growing, primary colonizers of

catfish ponds that might be present after the application of a broad-spectrum algaecide in which the abundance of the phytoplankton community is greatly reduced and then begins to recover. Diatoms often comprised a larger percentage of the population during the first 4–8 weeks of the study. In contrast, diatoms comprised a minor proportion of the phytoplankton population while chlorophytes and cyanobacteria co-dominated phytoplankton communities in a brackish water (Ray et al., 2010b) and a marine (Vinatea et al., 2010) BFT system. In our study, the addition of pond water to

Table 1

Divisions and designations (genus or species) of phytoplankton observed to be present during the study, number of tanks in which phytoplankton type was present, number of sampling dates on which phytoplankton type was present, and the abundance of single cells, colonies, or trichomes (natural units) in nine 15.6-m³ HDPE-lined biofloc technology (BFT) system culture tanks stocked with channel catfish initial biomasses ranging from 0.4 to 2.5 kg/m³.

Division	Genus or species	No. of tanks ^a	No. of dates ^b	Count ^c (natural units/mL)
Chlorophyta	<i>Ankistrodesmus</i> sp.	9	2–6	200–15,000 ^d
Chlorophyta	<i>Geminella</i> sp.	9	1–9	200–225,000 ^d
Chlorophyta	<i>Gloeocystis</i> sp.	9	1–4	200–380,000 ^d
Chlorophyta	<i>Pediastrum</i> sp.	9	1–9	200–24,250 ^d
Chlorophyta	<i>Scenedesmus</i> sp.	9	2–9	200–33,000 ^d
Cyanophyta	<i>Coelosphaerium</i> sp.	9	4–9	200–170,000 ^d
Cyanophyta	<i>Jaaginema subtilissimum</i>	9	7–11	200–112,500 ^d
Bacillariophyta	Centric diatoms	9	6–11	200–19,000 ^d
Bacillariophyta	Pennate diatoms	9	7–12	200–540,000 ^d
Cyanophyta	<i>Stigonema</i> sp.	8	1–2	400–9000 ^e
Euglenophyta	<i>Phacus</i> sp.	7	1–2	250–13,000 ^e
Chlorophyta	<i>Coelastrum</i> sp.	6	1–2	200–800
Euglenophyta	<i>Euglena</i> sp.	5	1–2	200–2500
Cyanophyta	<i>Anacystis cyanea</i>	4	1–2	200–13,600 ^e
Chlorophyta	<i>Protoderma</i> sp.	4	1	1600–9200 ^e
Chlorophyta	<i>Stigeoclonium</i> sp.	4	1	200–12,000
Cyanophyta	<i>Anacystis</i> sp.	3	1	200–400
Chlorophyta	<i>Actinastrum</i> sp.	1	1	6000
Chlorophyta	<i>Binuclearia</i> sp.	1	1	800
Chlorophyta	<i>Chlorella</i> sp.	1	1	200
Chlorophyta	<i>Closterium</i> sp.	1	1	250
Chlorophyta	<i>Dictyosphaerium</i> sp.	1	1	400
Chlorophyta	<i>Micractinium</i> sp.	1	1	200
Chlorophyta	<i>Oocystis</i> sp.	1	1	250
Chlorophyta	<i>Protococcus</i> sp.	1	1	200
Chlorophyta	<i>Ulothrix</i> sp.	1	1	200
Cyanophyta	<i>Aphanothece</i> sp.	1	1	3000
Cyanophyta	<i>Planktothrix agardhii</i>	1	1	200
Cyanophyta	<i>Phormidium</i> sp.	1	1	200
Cyanophyta	<i>Raphidiopsis</i> sp.	1	1	200
Cyanophyta	<i>Scytonema</i> sp.	1	1	400
Cyanophyta	<i>Tolypothrix</i> sp.	1	1	200
Bacillariophyta	<i>Melosira</i> sp.	1	1	200
Euglenophyta	<i>Trachelomonas</i> sp.	1	1	250

^a The number of tanks in which the phytoplankton type was observed to be present on at least one sampling date during the study.

^b The range of sampling dates (frequency of occurrence) on which the phytoplankton type was observed to be present in tanks during the study. There were 12 total sampling dates during the study.

^c Abundance as indicated by the count or range of counts of the phytoplankton type observed to be present in the tanks during the study.

^d More than 50% of the total phytoplankton count for at least one sample.

^e More than 25% of the total phytoplankton count for at least one sample. No superscript indicates never more than 25% of the phytoplankton count.

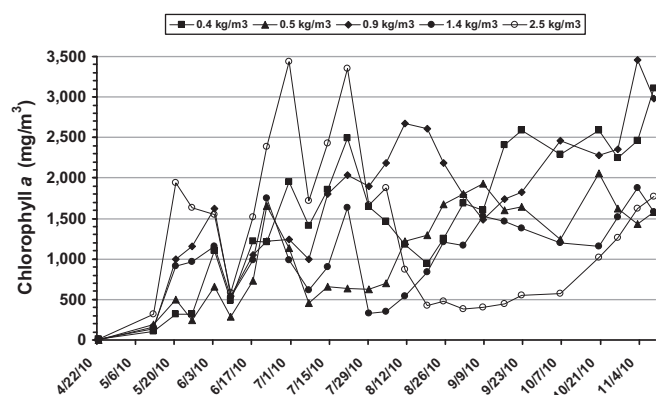


Fig. 2. Chlorophyll *a* concentration in biofloc technology (BFT) production system culture units (15.6 m³ HDPE-lined tanks) stocked with channel catfish at initial biomasses ranging from 0.4 to 2.5 kg/m³. Chlorophyll *a* concentrations for the 0.4 and 0.9 kg/m³ initial biomasses are the means of three tanks.

the tanks may have been beneficial in establishing communities of these preferred groups of phytoplankton in the biofloc tanks.

Overall, there was a gradual increase in phytoplankton biomass, as measured by chlorophyll *a* concentrations, throughout the study in most tanks, except for tank R8 which experienced a large decrease in phytoplankton biomass in late July from approximately 1900 mg chl *a*/m³ to less than 500 mg chl *a*/m³ (Fig. 2; Table 2). Mean chlorophyll *a* concentrations ranged from 1084 to 2015 mg/m³ and are indicative of high phytoplankton biomass (Table 2). In comparison, a study by Torrans (2005) determined chlorophyll *a* concentrations ranged from 342 to 439 mg/m³ in earthen ponds used for the intensive culture of channel catfish. Chlorophyll *a* concentrations in the present study were higher than those reported by Green (2010) for channel catfish production in BFT culture, likely because of the higher stocking and feeding rates in the present study. The increase in phytoplankton biomass can be beneficial in contributing to and maintaining dissolved oxygen levels in aquaculture systems, especially when the phytoplankton community composition is dominated by chlorophytes which are better oxygenators of the water compared to bloom-forming cyanobacteria and due to the faster growth rates of most eukaryotic types of phytoplankton. Bloom-forming cyanobacteria also have several other undesirable attributes including the following: (1) poor base for aquatic food chains; (2) the propensity to form blooms can lead to large shifts in dissolved oxygen levels in certain aquaculture systems (e.g., ponds) due to the rapid formation and die-offs of the cyanobacterial blooms; and (3) some species of cyanobacteria produce toxins and off-flavor compounds (Paerl and Tucker, 1995).

Net catfish yield increased linearly with increased stocking biomass ($y = 4.6951 + 1.7752x$, $R^2 = 0.688$) and was 5.8, 4.1, 6.5, 6.6, and 9.3 kg/m³ in tanks stocked with an initial fish biomass of 0.4, 0.5, 0.9, 1.4, and 2.5 kg/m³, respectively. Catfish net yields (kg/m³) for each tank were as follows: R1 = 4.1; R2 = 7.5; R3 = 6.1; R4 = 6.4; R5 = 5.3; R6 = 6.6; R7 = 5.7; R8 = 9.3; R9 = 6.0. Mean TAN and NO₂-N concentrations were low despite the high feed rates presumptively due to phytoplankton uptake and nitrification (Table 2). Mean NO₃-N, settleable solids, total suspended solids, and total volatile solids concentrations (Table 2) were comparable to values reported for freshwater (Green, 2010) and brackish water/marine (Ray et al., 2010a; Vinatea et al., 2010) BFT culture systems.

Geosmin levels in water collected from the biofloc tanks were relatively low throughout the study. On most sampling dates, geosmin levels in water were below the instrumental detection threshold of 1 ng/L (Table 3). The highest levels of geosmin in tank waters were present during the 6-22-10 to 7-14-10 sampling dates

Table 2
Mean (±S.D.) water quality variable concentrations in nine 15.6-m³ HDPE-lined biofloc technology (BFT) system culture tanks stocked with channel catfish initial biomasses ranging from 0.4 to 2.5 kg/m³.

Variable ^a	Tank (initial biomass)								
	R1 (0.5 kg/m ³)	R2 (0.9 kg/m ³)	R3 (0.4 kg/m ³)	R4 (0.9 kg/m ³)	R5 (0.4 kg/m ³)	R6 (1.4 kg/m ³)	R7 (0.9 kg/m ³)	R8 (2.5 kg/m ³)	R9 (0.4 kg/m ³)
Algae	20,854 (11,958)	32,812 (36,997)	15,625 (18,115)	30,367 (27,686)	18,810 (20,100)	52,167 (62,660)	105,417 (268,089)	69,317 (79,029)	43,142 (26,121)
Chl <i>a</i>	1257 (602)	1890 (1520)	1582 (1317)	1684 (967)	1446 (929)	1084 (451)	2015 (1766)	1224 (769)	2003 (1326)
NH ₄ -N	0.32 (0.52)	0.41 (0.60)	0.35 (0.44)	0.21 (0.15)	0.32 (0.42)	0.25 (0.17)	0.28 (0.18)	0.33 (0.25)	0.48 (0.94)
NO ₂ -N	1.32 (3.44)	1.13 (2.50)	0.69 (1.80)	0.76 (1.72)	0.58 (1.06)	1.70 (3.42)	0.70 (1.98)	1.34 (1.69)	0.97 (2.83)
NO ₃ -N	39.74 (34.48)	87.73 (75.20)	71.92 (65.33)	44.57 (43.79)	22.35 (16.74)	72.51 (68.76)	53.73 (53.74)	127.59 (99.70)	45.18 (36.30)
SS	27.9 (18.4)	48.5 (33.0)	49.2 (34.7)	37.4 (27.5)	28.8 (11.4)	36.8 (18.1)	40.5 (18.9)	65.5 (43.6)	38.4 (24.9)
TSS	339.27 (169.21)	524.36 (296.91)	523.89 (306.78)	477.01 (283.29)	350.08 (159.91)	453.71 (229.16)	500.99 (218.90)	604.28 (354.89)	426.60 (200.80)
TVS	254.28 (134.86)	410.90 (249.44)	402.89 (249.16)	368.95 (227.92)	271.94 (131.68)	357.42 (187.84)	385.48 (168.67)	479.82 (283.30)	310.19 (153.87)

^a Algae, natural units/mL; chlorophyll *a* (Chl *a*), mg/m³; total ammonia-nitrogen (NH₃-N), mg/L; nitrite-nitrogen (NO₂-N), mg/L; nitrate-nitrogen (NO₃-N), mg/L; settleable solids (SS), mL/L; total suspended solids (TSS), mg/L; total volatile solids (TVS), mg/L.

Table 3Mean (\pm S.D.) geosmin levels (ng/L) in water samples collected from nine different biofloc tanks during 2010.

Tank ^a	Sampling date											
	5-20	6-8	6-22	7-14	7-29	8-11	8-25	9-8	9-22	10-6	10-20	11-9
R1	1 (0)	25 (3)	20 (3)	0 (0)	0 (0)	7 (2)	1 (0)	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)
R2	1 (0)	2 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0)	69 (3)	59 (16)
R3	21 (5)	1 (0)	49 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	2 (0)	12 (1)
R4	0 (0)	14 (2)	15 (3)	5 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	25 (1)	24 (1)
R5	0 (0)	1 (0)	83 (24)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0)	22 (1)
R6	0 (0)	8 (0)	3 (2)	11 (2)	0 (0)	0 (0)	0 (0)	1 (0)	13 (2)	3 (0)	10 (0)	25 (1)
R7	0 (0)	0 (0)	13 (1)	54 (2)	6 (2)	5 (0)	6 (1)	1 (0)	1 (0)	15 (1)	6 (1)	1 (0)
R8	1 (0)	1 (0)	44 (5)	396 (76)	1 (0)	0 (0)	0 (0)	0 (0)	1 (0)	4 (0)	8 (0)	250 (19)
R9	1 (0)	1 (0)	20 (1)	4 (1)	1 (0)	1 (0)	0 (0)	1 (0)	2 (1)	1 (0)	3 (0)	6 (1)

^a Initial fish biomass: 0.4 kg/m³ (R3, R5, R9), 0.5 kg/m³ (R1), 0.9 kg/m³ (R2, R4, R7), 1.4 kg/m³ (R6), and 2.5 kg/m³ (R8).

Each mean was obtained from the analysis of a water sample in triplicate; S.D. = standard deviation. Values of "0" indicate levels were below the instrument detection threshold of 1 ng/L.

Table 4Mean (\pm S.E.) geosmin and 2-methylisoborneol (MIB) levels (ng/kg) in catfish fillet samples ($n=5$ per tank) collected from nine different biofloc tanks on 11-10-10.

	Tank ^a								
	R1	R2	R3	R4	R5	R6	R7	R8	R9
Geosmin	1.6 (0.3)	3.8 (0.2)	25.4 (2.9)	44.4 (8.4)	482.2 (94.9)	35.8 (4.6)	5.0 (0.7)	60.8 (11.4)	140.8 (17.6)
MIB	55.0 (3.7)	38.6 (5.9)	24.8 (3.1)	33.4 (7.0)	644.0 (91.4)	25.0 (1.4)	20.8 (1.6)	31.6 (4.6)	61.4 (8.1)

^a Initial fish biomass: 0.4 kg/m³ (R3, R5, R9), 0.5 kg/m³ (R1), 0.9 kg/m³ (R2, R4, R7), 1.4 kg/m³ (R6), and 2.5 kg/m³ (R8).

Each mean was obtained from the analysis of fillets from 5 separate catfish per tank and with the steam distillate from each fillet analyzed in triplicate; S.E. = standard error.

and 10-20-10 to 11-9-10 sampling dates, with the highest geosmin levels present in tank R8 of 396 ng/L on 7-14-10 and 250 ng/L on 11-9-10. These geosmin levels are far below those that can occur in aquaculture ponds in the southeastern United States, with geosmin levels in some pond waters exceeding 2000 ng/L (Schrader and Blevins, 1993; Zimba and Grimm, 2003).

Although some of the catfish harvested from each tank at the end of grow-out cycle contained geosmin in their flesh, the geosmin levels in most of these catfish were below 100 ng/kg (data not shown). Geosmin levels were highest in catfish from tanks R5 and R9, with mean levels of 482 ng/kg and 141 ng/kg (Table 4). The reported sensory detection threshold range for geosmin by trained processing plant flavor testers is 250–500 ng/kg (Grimm et al., 2004). The geosmin levels in the flesh of catfish from tank R5 ranged from 247 ng/kg to 752 ng/kg, and these catfish would likely have been designated as possessing an earthy off-flavor when evaluated by trained processing plant flavor testers. However, the geosmin levels in the flesh of catfish from tank R9 were 108–189 ng/kg, and therefore, it is unlikely these catfish would be found to be off-flavored by trained flavor testers.

Levels of MIB in the water of the 9 tanks were low throughout most of the sampling period (Table 5). Overall, MIB levels did not significantly rise until late in September and into October, with the

highest MIB levels detected in most tanks on 10-20-10. The highest MIB levels in tank waters were found to be present in tanks R5 (266 ng/L on 10-20-10 and 282 ng/L on 11-9-10) and R7 (278 ng/L on 10-20-10). However, these MIB levels were still well below those MIB levels that can be found in catfish aquaculture ponds in Alabama, Arkansas, Louisiana, and Mississippi where MIB levels can often exceed 700 ng/L (Schrader and Blevins, 1993; Schrader and Dennis, 2005; Zimba and Grimm, 2003).

Tank R5 was the only tank that yielded catfish with MIB levels in their flesh at sufficient levels to likely result in musty off-flavored catfish (Table 4). Levels of MIB in the fish collected from tank R5 ranged from 390 ng/kg to 932 ng/kg, and these levels are well above 200 ng/kg, a level attributed to be the sensory detection threshold value for the average trained flavor checker (Grimm et al., 2004). In summary, tank R5 was the only tank to yield catfish with geosmin and MIB at sufficient levels in the catfish flesh to likely result in off-flavor fish. Although tank R7 had high MIB levels (278 ng/L) in the water on 10-20-10 and the water in tank R8 had the highest geosmin levels (396 ng/L on 7-14-10 and 250 ng/L on 11-9-10) during the sampling period, the catfish from these tanks appeared to have purged potentially bioaccumulated MIB or geosmin from their flesh to levels below sensory detection threshold ranges by the time harvest of the crop was performed on 11-20-10.

Table 5Mean (\pm S.D.) 2-methylisoborneol (MIB) levels (ng/L) in water samples collected from nine different biofloc tanks during 2010.

Tank ^a	Sampling date											
	5-20	6-8	6-22	7-14	7-29	8-11	8-25	9-8	9-22	10-6	10-20	11-9
R1	4 (1)	3 (1)	3 (3)	6 (1)	3 (1)	5 (1)	6 (1)	3 (1)	4 (0)	5 (2)	5 (0)	3 (0)
R2	3 (1)	1 (0)	1 (0)	6 (1)	2 (1)	5 (2)	5 (1)	5 (1)	3 (0)	26 (3)	62 (9)	43 (8)
R3	3 (1)	2 (1)	1 (0)	3 (0)	0 (0)	4 (0)	4 (0)	5 (1)	6 (2)	7 (1)	5 (0)	5 (1)
R4	8 (4)	2 (1)	1 (0)	4 (0)	1 (1)	4 (0)	4 (1)	3 (1)	5 (1)	4 (0)	4 (1)	6 (1)
R5	7 (3)	1 (0)	0 (0)	2 (1)	0 (0)	4 (1)	4 (1)	3 (1)	6 (2)	59 (6)	266 (23)	282 (51)
R6	5 (1)	2 (0)	1 (0)	4 (1)	0 (0)	4 (1)	4 (2)	13 (4)	7 (3)	12 (3)	16 (2)	3 (1)
R7	3 (1)	1 (0)	3 (3)	2 (1)	2 (0)	8 (6)	4 (1)	8 (1)	46 (14)	90 (18)	278 (48)	6 (0)
R8	3 (1)	2 (0)	17 (5)	4 (2)	12 (4)	4 (1)	6 (1)	12 (3)	20 (3)	16 (1)	24 (1)	61 (10)
R9	1 (1)	1 (1)	5 (2)	2 (0)	4 (1)	8 (3)	3 (0)	4 (2)	5 (1)	6 (1)	17 (4)	27 (5)

^a Initial fish biomass: 0.4 kg/m³ (R3, R5, R9), 0.5 kg/m³ (R1), 0.9 kg/m³ (R2, R4, R7), 1.4 kg/m³ (R6), and 2.5 kg/m³ (R8).

Each mean was obtained from the analysis of a water sample in triplicate; S.D. = standard deviation. Values of "0" indicate levels were below the instrument detection threshold of 1 ng/L.

The results of correlation analysis found a positive relationship ($p=0.0009$) between chlorophyll *a* concentration and cumulative feed addition. This positive correlation is not surprising because a large input of manufactured feeds to catfish production ponds can result in high standing crops of phytoplankton (Boyd and Tucker, 1998). However, as nutrient loading rates increase, the diversity of the phytoplankton communities in catfish ponds will likely decrease (Reynolds, 1984). In our study, there was no clear pattern of a reduction in the diversity of phytoplankton communities at higher feeding rates (Fig. 1; Table 1). A positive relationship ($p=0.0011$) between cumulative feed addition and MIB concentrations in the water of the culture tanks was also found. However, no significant relationship was found between geosmin concentration and cumulative feed addition ($p>0.05$). In addition, no significant relationships ($p>0.05$) were found between the following sets of measured variables: (1) MIB concentration in the water and chlorophyll *a* concentration and (2) geosmin concentration in the water and chlorophyll *a* concentration. Although the correlation results are mixed in terms of the relationships of geosmin and MIB concentrations with cumulative feed addition, it appears that higher feeding rates may increase the potential for MIB-related off-flavor problems. However, our sampling of catfish to determine MIB concentrations in fillets at the end of the study found that the tanks with the highest daily feed rates (mean of 79.1 and 100.7 g/m³ per week for tanks R6 and R8, respectively) did not contain catfish with the highest MIB concentrations in their flesh (Table 4). It is likely that the MIB levels in the water of the tanks, uptake of MIB by the catfish, and depuration of MIB from the catfish flesh may not have been synchronized at the end of the study. Additional research in which catfish fillets are obtained during the weeks prior to harvest and analyzed for MIB concentrations may clarify this discrepancy.

None of the various genera and species of cyanobacteria listed in Table 1 has so far been conclusively identified as producers of geosmin or MIB. Although a recent report linked geosmin levels with the abundance of cell numbers of *Coelosphaerium kuetzingianum* in a freshwater lake, the researchers did not proceed to isolate this colonial cyanobacterium and detect geosmin production by the isolate in pure culture (Godo et al., 2011). Therefore, a species from this genus has yet to be confirmed as a producer of geosmin or MIB. In our study, there was no significant correlation between the numbers of natural units (colonies of cells) of the *Coelosphaerium* sp. and geosmin or MIB levels in water in the tanks. In fact, the greatest abundance of *Coelosphaerium* sp. observed in any of the tanks during the study was 170,000 natural units/mL and which comprised 98% of the phytoplankton community on the 8–25–10 sampling from tank R6 while no geosmin was detected in the same water sample (instrumental detection limit of 1 ng/L for geosmin).

Several species of centric and pennate diatoms were observed to be present in the tanks throughout the study, and these diatoms were most abundant and dominated (>50% of natural units) the phytoplankton communities in the tanks during the cooler spring and early summer months (Fig. 1). Several species of euglenophytes (division Euglenophyta), such as *Euglena* sp., *Phacus* sp., and *Trachelomonas* sp., were present in some of the tanks during the autumn months; however, these species were present in very low numbers relative to the entire phytoplankton community (less than 10% of phytoplankton community during most sampling events). Diatoms and euglenophytes have never been associated with incidences of geosmin and MIB off-flavor episodes in pond-raised catfish.

The attempts to isolate geosmin and MIB-producing actinomycetes yielded only one actinomycete isolate, and this isolate was presumptively identified to be a species in the genus *Nocardia* based upon colony morphology. This *Nocardia* sp. was determined via SPME–GC–MS analysis to not produce geosmin or MIB. Because these results demonstrate the presence of actinomycetes in the

biofloc tanks, this group of bacteria cannot be completely ruled out as potential contributors to geosmin and MIB in the tank water and subsequently in the catfish flesh.

The constant mixing and high turbulence of the water provided in the biofloc tanks may have inhibited the formation of blooms of relatively larger planktonic, filamentous species of cyanobacteria (e.g., *Raphidiopsis* spp., *Planktothrix* (*Oscillatoria*) *agardhii*, *P. perornata*) that can commonly occur and dominate phytoplankton communities in catfish production ponds (van der Ploeg and Tucker, 1993). In addition, the high turbidity of the water in the tanks may have reduced suitable conditions for the growth of planktonic, filamentous cyanobacteria due to the reduced availability of photosynthetically active radiation. During the study, *P. agardhii* was observed to be present once and in only one tank (R4) near the beginning of the study (5–20–10) at very low numbers (200 natural units/mL and comprising 0.5% of the phytoplankton community). The *Raphidiopsis* sp. was also observed to be present only once during the study and in one tank (R9) on the sampling date of 6–22–10 at very low numbers (200 natural units/mL and comprising 0.6% of the phytoplankton community). Water column turbulence can cause cyanobacterial cell and filament damage and disaggregation, disrupt beneficial microbial interactions that can adversely affect cyanobacterial growth, and result in direct competition of certain species of buoyancy-regulating cyanobacteria with eukaryotic phytoplankton for light due to the loss of the ability of the planktonic cyanobacteria to maintain an optimal vertical position in the water column (Paerl and Tucker, 1995). Eukaryotic phytoplankton such as diatoms and chlorophytes tend to proliferate under conditions of variable light and nutrient regimes. However, due to the consistently high nutrient loading rates (input) of the biofloc systems, the variable light conditions likely contributed more to the dominance of the phytoplankton communities by eukaryotic phytoplankton. Although *Jaaginema subtilissimum* is a filamentous species of cyanobacteria, it is very small (filament diameter of 1–1.5 µm) compared to some of the other previously mentioned cyanobacteria (e.g., *P. perornata* which typically has a filament diameter of 7–10 µm), and, therefore, it may be less susceptible to the small-scale shear of high rates of water turbulence. In addition, *J. subtilissimum* was often observed to be attached to suspended biofloc particles which may have provided protection from shear and assisted in providing adequate light and beneficial consortia interactions with bacteria present in the biofloc.

In summary, the BFT systems used in this study to culture channel catfish favored the development of phytoplankton communities dominated by small colonial types of cyanobacteria and small, fast-growing unicellular or small colonial types of green algae and diatoms. In addition, these BFT culture tanks were susceptible to episodes of geosmin and MIB in the tank waters and subsequent bioaccumulation of these compounds in the catfish flesh. The levels of geosmin and MIB in the tank waters were less intense and less persistent than episodes that can occur in catfish aquaculture ponds. Additional research is required to determine the microbial sources responsible for geosmin and MIB production in the BFT tanks and to further investigate the effects of high feeding rates on geosmin and MIB concentrations in the fillets of catfish cultured in BFT tanks.

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